Allergic asthma and bronchial hyperreactivity

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Summary

Asthma may affect 5-10% of the population in Western countries and is probably underdiagnosed (Mortagy et al., 1986). Despite improved possibilities for therapy and increased drug use morbidity and mortality are still increasing (Page, 1993). The social and financial burden of the disease is therefore substantial.

Bronchial hyperreactivity (BHR) is a prominent feature of the disease. It is defined as an increased reactivity of the airways to a wide range of physical, chemical and pharmacological stimuli. This "non-specific" bronchial hyperreactivity is an important determinant of the bronchial obstructive reactions that may develop after both allergenic and non-allergenic stimulation of the airways. Bronchial hyperreactivity may be induced by a number of exogenous factors including exposition to airborne allergen and is almost invariably associated with inflammation of the airways. Therefore, inflammation is generally considered as an important causative factor of (allergen-induced) bronchial hyperreactivity, although the exact mechanisms of inflammation-induced bronchial hyperreactivity are still unclear. Several changes in the airways could be involved, including an increased airway smooth muscle contractility, a reduction in airway calibre and a change in neurogenic, humoral or paracrine control of the airways (chapter 1).

For a critical evaluation of these possibilities anatomical, morphological and functional aspects of the airways are described in chapter 2.

Allergen provocation of allergic asthmatics results in airways obstruction through the release of bronchoconstrictive mediators released from mast cells. This early asthmatic reaction (EAR) lasts 1-2 hours and, in a number of individuals, is followed by a late asthmatic response (LAR) which develops between 4-6 hours after allergen exposure and may last for up to 24 h after the challenge. The LAR is characterized by an influx of inflammatory cells, predominantly eosinophils and neutrophils, into the airways, which is most likely initiated by the release of chemotactic factors by mast cells after allergen exposure. These inflammatory cells are potentially important for the development of allergen-induced BHR that may be observed before and after the late asthmatic response. However, the causal relationships between allergen-induced early and late phase asthmatic reactions, airway inflammation, and the development of BHR are largely unknown (chapter 3).

The aim of the studies described in this thesis was to elucidate some of the mechanisms involved in the development of allergen-induced BHR. To study these mechanisms, *in vivo* as well as *in vitro* investigations of airway functioning and inflammation are essential. For obvious reasons, most of these studies cannot be performed in human asthmatics. Therefore an animal model is required for these investigations. Many different animal models of allergic asthma have been developed, including rats, guinea pigs, rabbits, dogs, and sheep. For convenience, a small laboratory animal was preferred. Of

these, the ovalbumin-sensitized guinea pig may be in favour because of the well-documented allergic responses with respect to mediator release, early and late asthmatic reactions and airway inflammation, that closely mimic the responses observed in human asthmatics (chapter 4).

In order to investigate mechanisms of BHR in relation to the development of the LAR as well as the effects of pharmacological treatment upon these parameters, it was considered necessary to measure airway functions continuously for prolonged periods of time (24 h), and also to be able to repeat these measurements a number of times during several weeks. A thorough survey of available literature revealed that existing methods of airway function measurement are generally based on either (whole)body-plethysmography, and/or on invasive methods employing anesthesia. Since these methods did not meet our criteria we developed a novel method for the measurement of airway functions in permanently instrumented, conscious and unrestrained guinea pigs (chapter 5).

The technique utilizes a specially designed pneumotachograph or pitot tube that is placed inside the trachea. The pneumotachograph consists of a stainless steel cylinder with coaxial and perpendicular tubes attached to it, measuring the total and lateral pressure in the trachea, respectively. Via air-filled silicon tubes the pressures are fed into a differential pressure transducer, yielding a pressure difference proportional to the airflow inside the trachea. A small latex balloon was attached to a saline filled cannula and placed inside the thoracic cavity to measure pleural pressure using a second pressure transducer. The tubings used for both devices were subcutaneously driven to the neck of the animal and permanently attached. This enabled repeatable connection to the pressure transducers without the animals being aware of the measurements taken.

The airflow and pleural pressure measurements enabled calculation of tidal volume, dynamic compliance, and airway resistance, which were all in the range of values found with conventional methods. In this study, a very good correlation was observed between airway resistance and pleural pressure under normal as well as under obstructive conditions. To facilitate the ease of experiments and to ease the surgical burden on the animals, it was then decided to continue with sole pleural pressure measurement as an index of airway obstruction (chapter 5).

The sensitization and allergen provocation procedures applied to experimental animals may greatly determine the development of allergen-induced early and late phase reactions and BHR. Based on data available in literature our guinea pigs were initially sensitized to ovalbumin using two different adjuvants: Freunds Complete Adjuvant (FCA) to facilitate production of IgG_1-antibodies, and aluminum hydroxide ($Al(OH)_3$) to facilitate IgE levels. In both groups a significant airway obstruction was observed immediately after exposure to a low dose of antigen without prophylactic treatment with antihistaminics to prevent development of fatal anaphylaxis. The EAR lasted approximately 4 hours and was followed by a LAR in 4 out of 6 IgG animals and 8 out of 9 IgE animals. The LAR was

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variable in onset and duration, and was, therefore, determined as area under the time-pressure curve between 8 and 24 h after allergen exposure. Bronchial reactivity to histamine inhalation was measured as the provocation concentration causing a 100 % increase in pleural pressure compared to baseline (PC_{100}). A ratio in PC_{100} pre/post allergen exposure was determined after the EAR (6 h) as well as after the LAR (24 h).

In the IgG as well as the IgE animals allergen exposure resulted in a marked increase in bronchial reactivity to inhaled histamine at 6 h, which was lower, but still significantly increased at 24 h after provocation. Both in a qualitative and in a quantitative sense, the early and late increases in bronchial reactivity were very comparable to that observed in allergic asthmatics. In asthmatics, however, allergen-induced BHR may be sustained for several days or even weeks. Since IgE antibodies are predominantly involved in human asthma, the IgE animals were subjected to different allergen exposure protocols to investigate whether the late BHR could be enhanced and/or sustained. A second allergen provocation, 7 days after the first one, yielded a similar development of BHR. Daily repeated allergen provocations for four days resulted in a decreased development of BHR, despite a daily increase in allergen concentration necessary to reach identical early bronchospasm. In a final attempt to enhance late BHR, IgE-animals were resensitized twice (boostered) and subsequently exposed to allergen twice a week for 5 weeks. Since this protocol also failed to induce sustained BHR, it was concluded that this guinea pig model is an excellent model for early and late, but not for sustained BHR (chapter 6).

Approximately 70% of the animals developed a LAR after the EAR. When comparing these dual responding animals with single early responders with respect to the development of BHR, a clear difference was observed. While dual responders demonstrated a significant increase in the reactivity of the airways, before as well as after the LAR, such an increase was absent in single early responders. The presence of BHR before the LAR in dual responders might indicate that this early BHR may contribute to or even cause the development of the LAR. However, no significant correlation was observed between the BHR at 6 h and the severity of the LAR. In addition, in 2 animals responding to allergen exposure with only a late reaction, an increase in bronchial reactivity before the LAR was absent, indicating that, although the presence of BHR may increase the severity of the LAR, these two features of asthma are causally unrelated and merely triggered by the same event, i.e. allergen exposure to sensitized animals. This conclusion is supported by the marked correlation between the initial increase in pleural pressure during the EAR and the early as well as late BHR, indicating that early events during the EAR may determine the subsequent development of BHR.

The observation that the early BHR is greater in magnitude that the late BHR, in combination with the absence of late BHR in the single late responders, suggests that late BHR is not caused by the LAR as was currently believed, but merely a remainder of the early BHR. As in human studies, this hypothesis is confirmed by the absence of a

significant correlation between the severity of the LAR and the degree of BHR after the LAR.

Since numerous studies have indicated a close association between airway inflammation and BHR in asthmatics, it is generally accepted that inflammatory cells and their mediators play an important role in the allergen-induced LAR as well as BHR. The number of eosinophils and neutrophils in the bronchoalveolar lavage (BAL)-fluid was markedly enhanced after the EAR as well as the LAR, similar to that observed in asthmatic patients. However, a dissociation was observed between the time course of eosinophil influx, which was maximal at 24 h after allergen exposure, and the development of BHR, which was maximal at 6 h (chapter 7).

A possible explanation for this dissociation may be the activation state of the infiltrated eosinophils. Indeed, using eosinophil peroxidase (EPO) activity in the BAL-fluid as a marker for eosinophil activation, an increase in activation state was observed at 6 h after allergen exposure, while EPO-activity was not further increased at 24 h. Surprisingly, the EPO-activity was closely correlated with BHR at 24 h, but not at 6 h after allergen exposure. It is therefore likely that additional mechanisms contribute to the early BHR. Using the same guinea pig model, a dysfunction of autoinhibitory muscarinic M_2-receptors has recently been observed in our laboratory, contributing to the BHR at 6 h but not at 24 h after allergen exposure (Ten Berge et al., 1992). This dysfunction is presumably caused by allosteric antagonism of major basic protein, another positively charged eosinophil derived protein, on the M_2-receptor (Jacoby et al., 1993) (chapter 8).

Since the onset of allergen-induced bronchospasm as well as its recovery by bronchodilators may occur very rapidly, airway smooth muscle contraction is clearly implicated in the pathogenesis of allergic asthma. Therefore, changes in airway smooth muscle function could contribute to allergen-induced BHR. Contractile and relaxant responses of tracheal smooth muscle preparations from allergen-challenged guinea pigs were investigated *in vitro*. The sensitivity and maximal response to methacholine and histamine were not altered at 6 h and 24 h after a single allergen exposure as well as at 24 h after four daily repeated provocations. By contrast, a small but progressive loss of ß-adrenoceptor sensitivity, but not of maximal relaxation, was observed after the allergen provocation, which was parallelled by an increase in the number of eosinophils in the BAL-fluid. These results suggest that (mediators from) eosinophils may be involved in the altered ß-adrenoceptor sensitivity. Since the time course of ß-adrenoceptor dysfunction, increasing in time, was strongly dissociated from the development of BHR, being maximal shortly after allergen exposure, this reduced ß-adrenoceptor function is not regarded as principally important in the development of increased airway reactivity. Its relevance for the use of bronchodilator treatment, however, is unknown (chapter 9).

Since allergen-induced EAR and LAR, as well as early and late BHR and airway inflammation proved to be very reproducable in guinea pigs when the allergen exposure is

repeated with a weekly interval, we were able to study the effects of various pharmacological compounds on these features of asthma. Since we found that the development of the LAR as well as BHR in this model appeared to be critically dependent upon the initial degree of airway obstruction during the EAR, which is predominantly caused by the release of histamine, we investigated the effect of the selective H_1-antagonist mepyramine on allergen-induced asthmatic reactions, BHR and inflammatory cell infiltration (chapter 10). We observed that inhalation of mepyramine 1 h before allergen challenge caused a significant reduction in the severity of the EAR which can easily be explained by H_1-antagonism. In addition, we found that mepyramine caused a significant reduction in the magnitude of the LAR and prevented the early as well as the late BHR to both histamine and methacholine. Because in control animals mepyramine hardly reduced histamine-induced bronchoconstriction at 7 h after application, it is unlikely that the inhibition of the LAR as well as the early and late BHR to histamine and methacholine proceed via a direct effect of the anti-histaminic on airway smooth muscle. The effects of mepyramine on both the LAR and BHR could, however be due to a reduction of airway inflammation, as indicated by a reduced number of infiltrated cells in the BAL-fluid. The results of this study further indicate that profylactic administration of antihistaminics to prevent anaphylaxis in allergen-challenged guinea pigs, as described previously, will reduce the development of the LAR and BHR in these animals.

The possible contribution of an enhanced vagal reflex mechanism to the severity of BHR was investigated using the anticholinergic compound ipratropium bromide to prevent cholinergic bronchoconstriction. A contribution of this vagal reflex mechanism was indicated by the observation that BHR to histamine inhalation was higher than to methacholine, the latter inducing only direct airway smooth muscle contraction. The inhibition of allergen-induced early BHR to histamine by ipratropium, in a concentration which had no effect on histamine-induced bronchoconstriction in control animals, clearly indicated that the vagal reflex bronchoconstriction is increased after allergen exposure. Possible mechanisms for the enhanced vagal reflex bronchoconstriction may include disruption of the airway epithelium by cytotoxic cationic proteins released from eosinophils, thereby facilitating the access of histamine to sensory nerve endings as well as a dysfunction of the autoinhibitory muscarinic M_2-receptors, possibly by the same cationic proteins. A 10-fold increase of the ipratropium concentration, however, did not further reduce bronchial reactivity to histamine, indicating that other mechanisms also contribute to the early BHR (chapter 11).

Airway inflammation is clearly involved in the pathogenesis of asthma. Nonetheless, symptomatic bronchodilator therapy is still widely applied in asthma and this may underlie the ever increasing morbidity and mortality from this disease.

Ideally, bronchodilator therapy should also express anti-inflammatory activity, beyond mast cell stabilization. Current evidence suggests that phosphodiesterase (PDE) inhibitors

may combine the bronchorelaxant, mast cell stabilizing, and anti-inflammatory actions necessary for adequate treatment. Especially the newer type IV-selective PDE-inhibitors may be useful because they specifically inhibit type IV-PDE present in airway smooth muscle as well as in inflammatory cells and are likely to express reduced side-effects.
The non-selective PDE-inhibitor theophylline, as well as the type IV-selective PDE-inhibitors rolipram and ORG 20241 were used in concentrations that did not affect the magnitude of the early and late phase response. When administered 1 h before allergen provocation all three compounds, at these concentrations, significantly reduced allergen-induced early and late BHR to a similar degree. In addition, BAL-studies indicated a selective inhibition of airway inflammation by the PDE-inhibitors. Thus, theophylline significantly reduced the allergen-induced influx of eosinophils, neutrophils, and macrophages. Rolipram reduced the number of neutrophils and lymphocytes, while the number of eosinophils and macrophages were reduced by ORG 20241. The results suggest that low doses of the PDE-inhibitors markedly reduce allergen-induced development of BHR by inhibition of inflammatory cell infiltration. Moreover, these results indicate that BHR is not due to the actions of a singular cell type but the result of a carefully orchestrated series of events, involving several cell types.

In conclusion, allergen-induced early and late phase asthmatic responses, early and late phase bronchial hyperreactivity and airway inflammation in this newly developed guinea pig model of allergic asthma are strikingly similar to those observed in human asthma. The development of BHR and LAR appear to be principally independent, although the early BHR may enhance the severity of the LAR. Both features of allergic asthma are initiated during the early phase of the allergic cascade. Histamine is a very important mediator in the EAR, possibly through a facilitatory action on the infiltration of inflammatory cells into the airways. The higher BHR for histamine, compared to that for methacholine, suggests a contribution of a cholinergic reflex mechanism. Using ipratropium bromide it was observed that sensory nerve endings, which activate the vagal reflex bronchoconstriction, are indeed more sensitive to histamine after allergen provocation and thus may contribute to the early BHR. Activation of infiltrated eosinophils may contribute to this enhanced sensitivity by the release of cytotoxic mediators, which can damage the epithelial lining. The markedly increased EPO-activity in the BAL-fluid supports this hypothesis. Changes in airway smooth muscle function do not substantially contribute to allergen-induced BHR, although a small time-dependent decrease in ß-adrenoceptor sensitivity was observed after allergen provocation.
The importance of inflammatory cells for the development of allergen-induced BHR is also supported by the effects of mepyramine and the phosphodiesterase-inhibitors theophylline, rolipram and ORG 20241, which more or less selectively inhibited the influx of inflammatory cells into the airways and therewith reduced BHR.